Original Article

The Effect of Intraperitoneal Administration of Oleuropein on Pentylenetetrazole Induced Epilepsy in Mail Rat

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Abstract

Background & Objective: Epilepsy is one of the most common neurological disorders in humans, which appears as sudden, episodic, repetitive, and unpredictable seizures, and these patients suffer from memory impairment. Medicinal herbs have long been used to treat epilepsy. In this study, the impact of effective oleuropein on the time of arising of attacks and the rate of attack's incidence of tonic, clonic, tonic-clonic, and total seizure longevity in male rats was investigated.
Materials & Methods: Forty rats were randomly divided into four groups of 10. The negative control group (the recipient of normal saline), the positive control group (the recipient of diazepam 1 mg/kg), and the two treatment groups (the recipient of doses of 10 and 20 mg/kg of oleuropein). After 30 min of oleuropein administration, saline, or diazepam, pentylenetetrazole was injected intraperitoneally into rats' bodies at a dose of 85 mg/kg, and 30 min after injection, seizure parameters were evaluated. Data were analyzed by one-way ANOVA and Tukey's statistical tests.
Results: Injection of the oleuropein significantly increased the threshold of seizures in the form of initial delay at the arising of seizure in the positive control group and treatment groups ($P< 0.001$). Furthermore, it reduces the time of seizures in different phases ($P< 0.001$). In addition, a dose of 10 mg/kg oleuropein has the highest effect on total seizure longevity.
Conclusion: This study showed that oleuropein has an appropriate anti-seizure effect and future studies appear to be necessary for further understanding of the mechanism.

Keywords: Oleuropein, seizure, PTZ

Introduction

Epilepsy is a common neurological disorder, which has been defined as repetitive seizures. In most patients, epilepsy can be controlled via taking medicines, and only about one-third of these patients resist medicine. Frequent convulsions/seizures are considered a risk factor for early death in epilepsy (1). Pentylenetetrazole (PTZ) is one of the Gaba-aminobutyric acid (GABA) receptor antagonists, which can cause severe seizures (2). In the seizure created by the GABA receptor antagonist, Pentylenetetrazol is used for analog of created seizures of Petit mal yet, but studies show that PTZ competitively antagonizes the receptor of GABA-A, which exists in the neuronal membrane of the central kind and general seizures (1) in humans (3)

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and generalized seizures in rodents. High repeatability and providing a basis to compare different anti-seizure chemical compounds under standard conditions are among the benefits of this medicine (4). The primary mechanism of PTZ-induced seizure is not completely known yet, but studies show that PTZ competitively antagonizes the receptor of GABA-A, which exists in the neuronal membrane of the central nervous system. Possibly through allosteric interference with this receptor and the chloride ion flow created by GABA-A inhibits, or in other words, PTZ was blocked by the chloride ionophores connected to the receptor of GABA-A, and can induce seizure (5). Additionally, GABA receptors have connection points with medicines such as benzodiazepines, which can increase the open duration of chlorine channels and apply anti-seizure and sedative effects (6). Research shows that, oxidative stress stimulates the brain to create seizure attacks (7). The creation of oxidative stress is an imbalance between the release of oxygen species, nitrogen, and the production of body defense materials, such as antioxidants. The increase in reactive oxygen species leads to oxidative stress. (8). Species of reactive oxygen (free radicals) can damage all biomolecules, including lipids, proteins, and DNA. The body inactivates all types of free radicals using antioxidants (9). Over recent years, natural substances with nervous protection and antioxidant have taken top priority in treating common neurological diseases (10). Phenolic compounds and flavonoids are among the plants' most critical natural antioxidant sources, and many therapeutic effects have been attributed to them (11). In recent reviews, the possible effect of flavonoids on the central nervous system has been observed, where flavonoids, by binding to benzodiazepine receptors have the same effects as benzodiazepines. This conclusion was based on the evidence, indicating the effect of flavonoids on somnolence creation, anxiety removal, and seizure control. The activation of benzodiazepine receptors via strengthening the brain's GABAergic system can stop epileptic attacks. Oleuropein is the most important phenolic compound of olive leaf and is the factor behind the exceptional bitter taste of the olive fruit, which has several pharmacologic properties. So far, it has been observed that this combination has antioxidant, anti-inflammatory, antiatherogenic, antimicrobial, and antiviral properties (12, 13). The role of oxidative stress in neurodegenerative diseases and the proven antioxidant effects of oleuropein in different tissues of rats (14, 15) has been proved. Considering different seizure phases, oleuropein's effect on these phases' improvement has not been studied so far. Therefore, this study investigates the effect of oleuropein on improvement of epilepsy in the PTZ-induced seizures model.

Materials & Methods

Research Methodology

In this experimental study, 40 male Wistar rats with an approximate weight of 250-200 g were used. The rats were randomly divided into 4 groups of 10. Animals had free access to water and food during maintenance except during experiments. The diet rats included ready-made meals and refined urban water. The rats had a cycle of 12 h of darkness and 12 h of brightness and laboratory temperature at the limit of 22±2 °C. They sorted them into 4 groups, including the negative control group, the positive control group (recipient diazepam as an anticonvulsant medicine at a dose of 1 mg/kg), and 2 treatment groups (recipient Oleuropein at doses of 10 and 20 mg/kg). Thirty minutes after injection of these materials, PTZ (Sigma Co.) intraperitoneally at a dose of 85 mg / kg was injected, and animals seizure behaviors, including latency at the arising of seizure, duration of tonic seizure, duration of clonic seizure, duration of tonic-clonic seizure, and total seizure longevity, were recorded immediately and for the next 30 minutes.

Data Analysis

After data collection, data were statistically analyzed using SPSS 17 software, one-way analysis of variance (ANOVA), and the Tukey's test. Results are expressed as the

mean ± standard deviation. A p value less than 0.05 was considered as significant.

Result

In this section, oleuropein with doses of 10 mg and 20 mg/kg body weight of rats was investigated in the treatment of epileptic seizures, which was induced by PTZ. Data proved that the medicine used in each dose significantly affected the zero stage of seizure in time delay increasing of seizure arising (P <0.001) in the comparison between treatment groups and the negative control group. In addition, a significant increase (P <0.05) was observed in the comparison between each of the treatment groups with the positive control group (diazepam) (chart 1). The results of the mean tonic seizure time in the treatment groups with both doses of medicine showed a reduction in comparison with the negative control group. Additionally, a greater reduction was observed at a dose of 10 mg/kg compared to a dose of 20 mg/kg (chart 2). In the clonic seizure stage, both treatment groups showed a significant duration reduction compared to the negative control group (P<0.001) (chart 3). In the third seizure phase, that is, the mean of tonic-clonic seizure time, both treatment groups with different doses of Oleuropein showed a significant reduction compared to the negative control group (P<0.001) (chart 4). Finally, the results of the comparison of the mean total of the seizure time between experimental groups showed that the treatment group with a 10 mg/kg dose of oleuropein had a similar function to the positive control group. Moreover, a significant reduction compared to the negative control group was revealed, and treatment group 2 with a dose of 20 mg/kg showed a significant reduction compared to the negative control group (P<0.001) (chart 5).

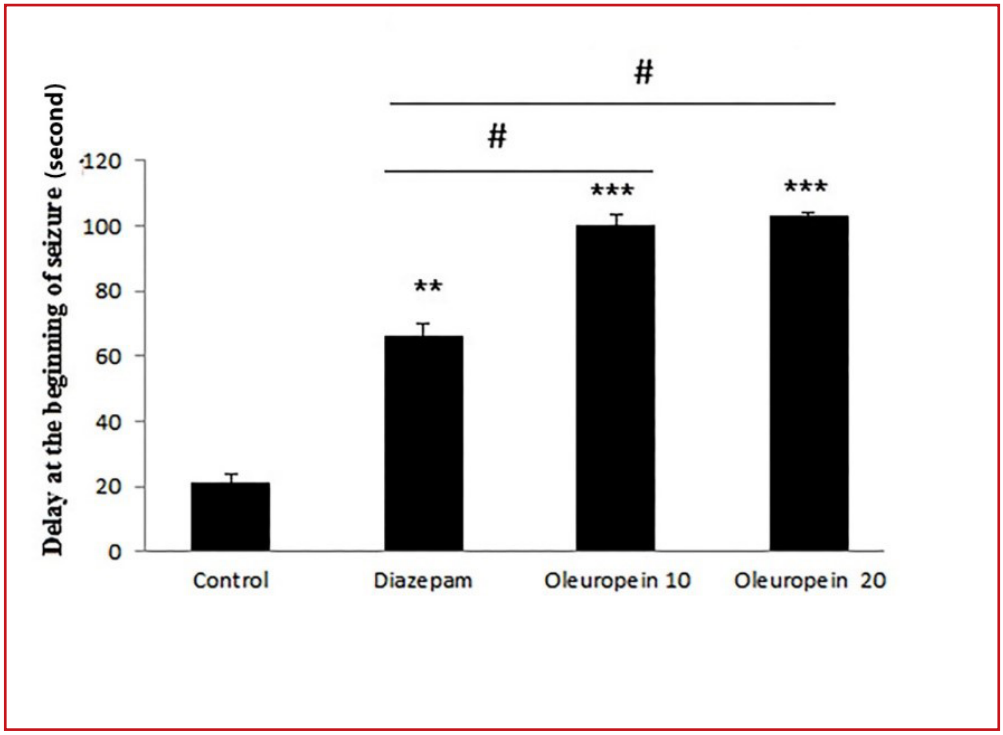

Chart 1. Comparison of the mean time required to start of seizure (per second) in the 4 groups under test (the asterisk (*) denotes the significant difference with control group, and *** indicates P<0.001) (n=10)

Control = Saline

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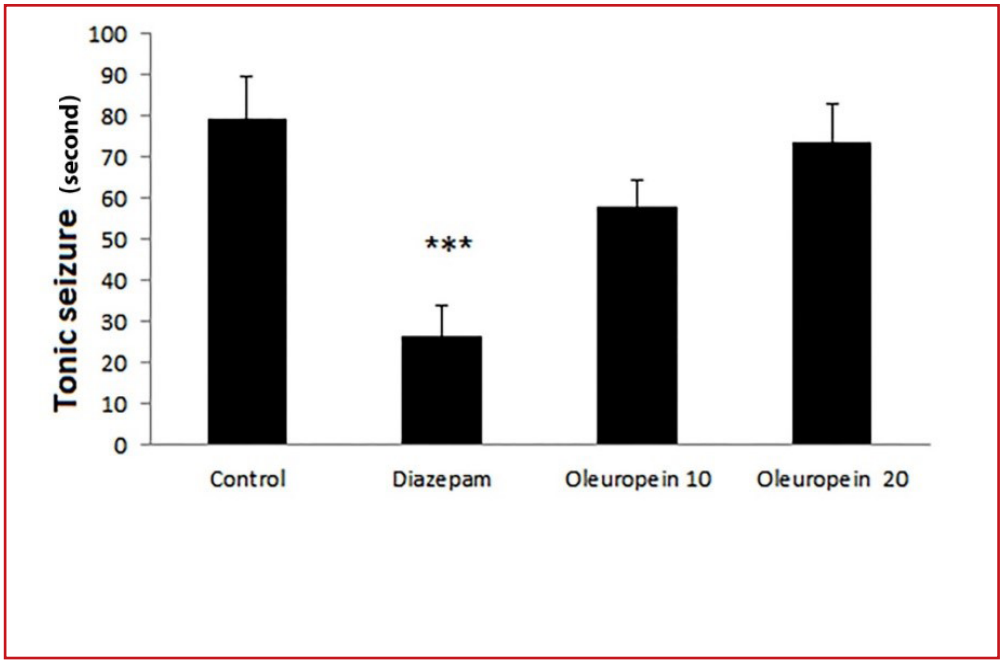

Chart 2. Comparison of the mean time of tonic seizure incidence (per second) in the 4 groups under study (the asterisk (*) denotes the significant difference with control group, and *** mark indicates P<0.001) (n=10)

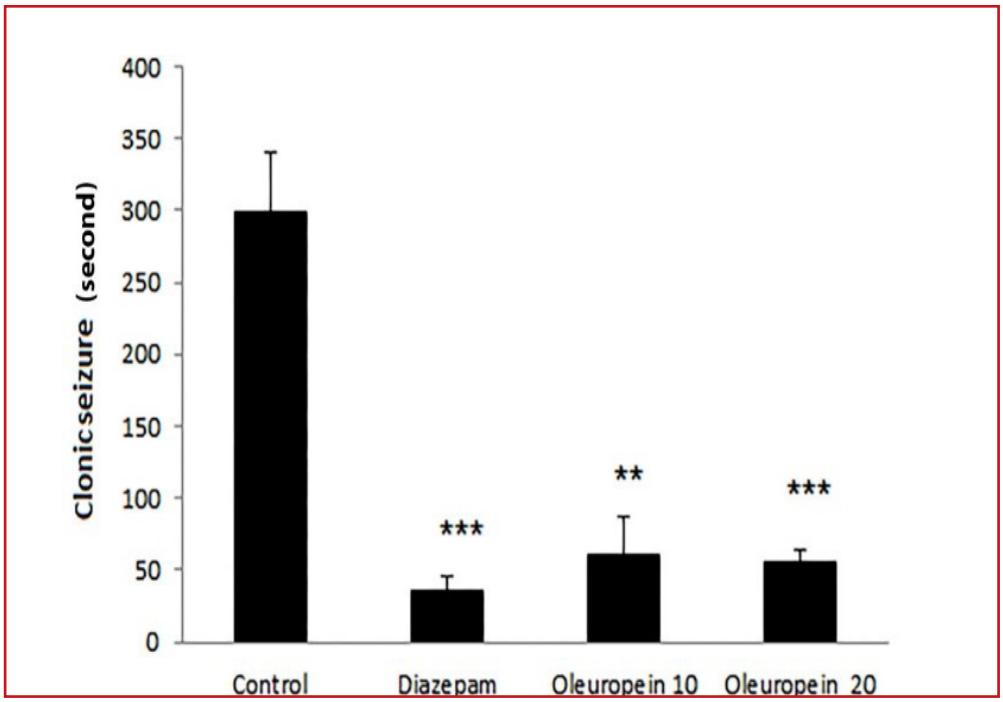

Chart 3. Comparison of the mean time of clonic seizure incidence (per second) in the 4 groups under study (the asterisk (*) denotes the significant difference with control group, and *** mark indicates P<0.001) (n=10)

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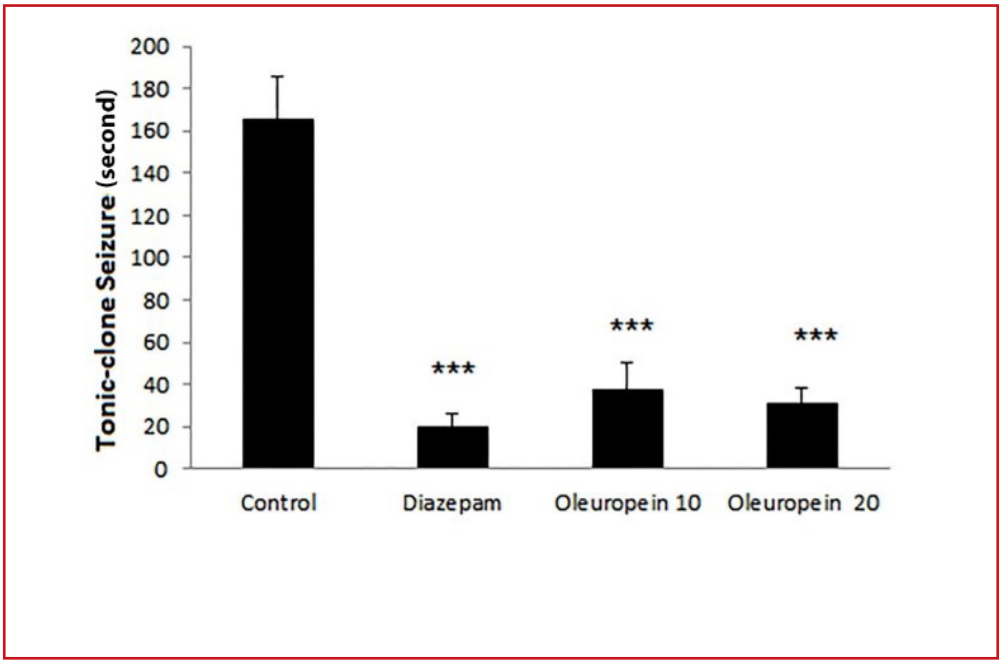

Chart 4. Comparison of the mean time of tonic-clonic seizure incidence (per second) in the 4 groups under study (the asterisk (*) denotes the significant difference with control group, and *** mark indicates P<0.001) (n=10)

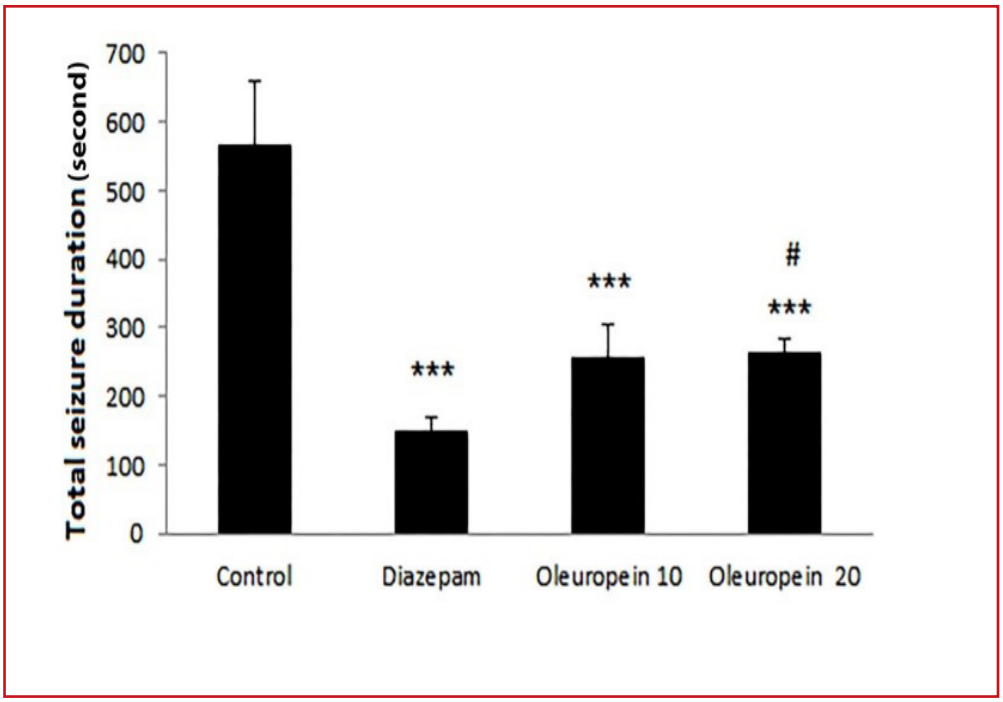

Chart 5. Comparison of the mean of total time of seizure (per second) in the 4 groups under study (the asterisk (*) denotes the significant difference with control group, and *** mark indicates P<0.001) (n=10)

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Discussion

In this research, injection of oleuropein with a dose of 10 mg/kg as the best dose, had a performance like positive control group (diazepam) and can with a performance like a diazepam, increase the rate of GABA and affect PTZ-induced seizure by intensifying inhibitory trends and reducing excitatory transmission. GABA is one of the neutral amino acids with inhibitory effects in the mammalian brain. Disruption in inhibitory mechanisms has long been known as a justification for initiating epileptic seizures. The GABAergic neurons form an additional part of the communication between the neurons in the hippocampus and cortex of the brain (16). Several studies have shown that medicines that reduce GABA concentrations or block GABA receptors cause seizures in different species of laboratory animals, while medicines that increase GABA levels or improve GABA transmission have anti-seizure effects (17). Research studies have shown that antioxidants can boost the stability of cell membranes and cause an increase in the resistance of neurons against oxidative damage, and increase the brain's antioxidant capacity against oxidative damage (18). The studies conducted over the past years have proved the role of cyclooxygenase enzyme in PTZ- induced seizure (19). It seems that the inhibition of cyclooxygenase-2 by the inhibition of prostaglandin E2 (by reducing liberation of glutamate) can decrease epilepsy (20). Because the activation of cyclooxygenase-2 causes an increase in free radicals synthesis, it leads to oxidative stress and GABAergic neurons apoptosis. Therefore, the concentration of glutamate has increased due to the removal of GABA inhibitory effect from glutamatergic neurons, and this process increases glutamatergic tone in neurons and neuronal network. Eventually, it increases the severity of the seizure, and it has been proved that oleuropein reduces the expression of cyclooxygenase-2 (21) and it has been reported as a free radical Sweeper (22). Moreover, according to our previous studies, the cyclooxygenase enzyme inhibitors such as rofecoxib, aspirincetoprofen, and flunixin meglumine inhibit seizures, and consequently, the increased expression of cyclooxygenase enzyme can induce epileptic seizures because of inflammation (23).

Conclusion

Though this research showed that oleuropein stops none of the different phases of seizure completely, it affected the reduction of the duration of seizures in different phases and the rate of death due to repeated seizures. In addition to affecting the different phases of seizure, oleuropein can be effective by increasing seizure threshold. The role of this substance in preventing oxidative stress can be one of the effective mechanisms in these mentioned effects. Additionally, having a performance like benzodiazepines (diazepam in this research), oleuropein can increase the amount of GABA and improve GABA transmission and, by inhibiting cyclooxygenase-2 in the PTZ-induced seizure model, it has anti-seizure effects. Of course, to determine this medicinal plant's effect on general epilepsy in humans, more serious, accurate, and clinical studies are needed.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

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